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Glomerular C3c Localization Indicates Ongoing Immune Deposit Formation and Complement Activation in Experimental Glomerulonephritis

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In antibody-mediated glomerular disease, deposits of C3 (C3b) are common and are degraded by factor I to C3c and C3d. However, the kinetics of C3b degradation in glomerulonephritis have not been defined. To do this, we studied three models of complement-dependent glomerulonephritis with established C3 deposits (passive Heymann nephritis, cationized immunoglobulin G membranous nephropathy, and concanavalin A–anti-concanavalin A glomerulonephritis). C3b deposition was halted by administration of cobra venom factor, and the disappearance of C3c and C3d from glomeruli was measured with specific antibodies and quantitative fluorescence densitometry. Results showed that C3c deposits were reduced by over 85% within 24 hours in all three models. C3c clearance was unaffected by site or mechanism of deposit formation. C3d deposits persisted despite lack of ongoing complement activation. In passive Heymann nephritis when disease activity was monitored by urinary C5b-9 excretion, C3c was cleared in parallel with return of urine C5b-9 excretion to normal values. We conclude that glomerular deposits of C3c are cleared within 24 hours of cessation of complement activation. Positive staining for C3 utilizing antibody specific for the C3c portion documents recent complement activation usually reflecting new immune deposit formation. (Am J Pathol 1993, 142:179–187)

Many forms of immune renal disease are characterized by deposition of immunoglobulin (Ig) and complement components at various sites within the glomerulus.[1] Antibody-induced glomerular injury is mediated primarily by complement, either through generation of complement-derived chemotactic peptides that recruit inflammatory effector cells or through sublytic injury to resident glomerular cells from membrane-inserted C5b-9.[1–3] Glomerular complement activation by classical or alternate pathways is regulated primarily by activation, recruitment, and inactivation of C3.[4] Cleavage of C3 by a C3 convertase results in formation of the anaphylatoxin C3a and C3b. C3b is able to bind covalently to cell membranes and other structures.[5] Bound C3b generates a C3 and C5 convertase unless it is cleaved by factor I to form inactivated C3b (C3bi). In a second proteolytic step, C3bi is cleaved by factor I to C3c, a biologically inert peptide which dissociates rapidly from the binding site, and C3d, which remains covalently bound.[4–7] Thus, the presence of C3c may be a marker of recent complement activation, whereas C3d may persist in tissue long after complement activation ceases.

We reasoned that detection of C3 using an antibody specific for C3c might provide a useful index of immune glomerular disease activity when activity is defined as ongoing immune deposit formation accompanied by C3 activation. We tested this hypothesis in three different models of complement-mediated glomerulonephritis using an antibody specific for C3c. In each model, the kinetics of C3b degradation and C3c disappearance were quantitated when complement activation was abrogated with cobra venom factor (CVF) and degradation by factor I was allowed to proceed. We further utilized urinary excretion of C5b-9, a recently established marker of ongoing immune deposit formation and C5b-9 insertion in the glomerular epithelial cell in the Heymann models of membranous nephropathy,[8–10] to correlate the presence of C3c in glomeruli with active ongoing immune deposit formation.

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Our results indicate that glomerular C3c deposits are cleared quickly from the glomerulus when deposit formation and/or complement activation ceases and that C3c deposits correlate closely with ongoing immune deposit formation and complement activation. These findings provide support for the hypothesis that glomerular deposition of C3, as detected by most commercially available anti-C3 antibodies which are reactive primarily with C3c, may serve as a sensitive marker of the activity of antibody and complement-mediated glomerular diseases.

Materials and Methods

Experimental Design

The kinetics of C3b inactivation by factor I cleavage *in vivo* were assessed by quantitative immunofluorescence (IF) densitometry for C3c and C3d in three models of complement-dependent immune complex glomerulonephritis (GN): passive Heymann nephritis (PHN), concanavalin A (con A)–anti-con A, and cationized IgG anti-IgG nephritis (cat-IgG). In each model, the presence of IgG and C3 was confirmed by biopsy. Complement depletion was then induced with CVF to prevent continued C3b deposition while allowing degradation of previously bound C3b by factor I to proceed. C3b disappearance was assessed 24 hours later by quantitative IF densitometry with antibodies specific for C3c and C3d. The results were compared with biopsies obtained before CVF treatment and to controls that were not complement-depleted. In PHN, ongoing complement activation was halted in separate groups 2, 4, and 7 days after disease induction, and glomerular C3c and C3d deposits were quantitated in each group 24 hours later. In the cat-IgG and con A models of GN, complement activation was abrogated with CVF at 24 hours after disease induction, and biopsies were obtained 24 hours later.

In a separate study, IF densitometry for C3c and C3d was carried out in a large group of unmanipulated PHN rats. Groups of three rats each were biopsied at 3-day intervals for 23 days. Densitometric readings were correlated with urinary C5b-9 excretion, a previously established marker of immunological disease activity in this model,[8,9] and urine protein excretion.

Characterization of the Fluorescein Isothiocyanate (FITC)–Labeled Antibodies to Rat C3c and Human C3d

The specificity of the polyclonal antibodies to rat C3c and human C3d for the cleavage fragments of rat C3 were defined by immunoblot analysis. Rat ethylenediamine tetraacetic acid-(EDTA)–plasma and rat serum in which C3 was activated by incubation with 0.5 units CVF/ml for 48 hours at 37 C, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to nitrocellulose membranes. After blocking with 1% gelatin in phosphate-buffered saline, pH 7.2 (PBS) for 1 hour, the membranes were incubated with goat anti-rat C3 FITC-IgG (Cappel, Malvern, PA) or with rabbit anti-human C3d FITC-IgG (Dako, Santa Barbara, CA) for 1 hour. The membranes were then washed with PBS followed by incubation with either biotinylated rabbit anti-goat IgG (Zymed, San Francisco, CA) or biotinylated sheep anti-rabbit IgG (Amersham, Arlington Heights, IL). Bound antibody was detected using strepavidin-peroxidase (Amersham) and the peroxidase substrate 4-chloro-napthole (Sigma Chemical Company, St. Louis, MO) as described previously.[8,10] Molecular weights were estimated using prestained standards (BRL, Gaithersburg, MD).

Induction of Experimental Glomerulonephritis

Three types of experimental GN were induced in 200-g male Sprague-Dawley rats (Tyler Laboratories, Bellevue, WA). PHN was induced in 18 rats by injection of 25 mg of sheep anti-Fx1A IgG prepared as described elsewhere.[11] On days 2, 4, and 7, groups of six rats underwent renal biopsy and four randomly selected rats in each group had ongoing complement activation halted with CVF. The two remaining rats served as untreated controls. Biopsies were repeated 24 hours later in CVF-treated and untreated groups. Cationized IgG–anti-IgG subepithelial GN was induced in six rats after removal of the left kidney. The right kidney was perfused via the superior mesenteric artery with 125 μg of human cationized IgG as described elsewhere.[12] Thirty minutes after renal artery perfusion, the rats were injected intravenously with 0.9 ml of heat-inactivated rabbit antiserum to human IgG.[12] Rats were biopsied 24 hours later and randomized as above to receive CVF. Biopsies were repeated 24 hours later in CVF-treated and untreated rats. Con A anti-con A subendothelial immune complex nephritis was induced by renal artery perfusion of 125 μg of con A (Miles, Naperville, IL) followed by 18 mg of rabbit anti-con A IgG as described previously.[13] Twenty-four hours later, rats were biopsied and then randomized to two groups as above. A second biopsy was performed at sacrifice 24 hours after CVF treatment.

Cobra Venom Factor Depletion of Plasma C3

Complement was depleted by intraperitoneal administration of CVF (Naja Kuonthia, Cordis, Miami, FL) as described elsewhere.[8,9] Twenty-four hours after injection of CVF, whole blood was collected into syringes containing EDTA at a final concentration of 60 mmol/L EDTA, and EDTA–plasma was separated by centrifugation. Plasma C3 levels were measured by radial immunodiffusion using polyclonal antibody to rat C3.[14] The assay was standardized with a pooled plasma sample from four normal rats which was arbitrarily assigned a value of 100%. All rats treated with CVF had C3 levels below 10% of normal values at the time of sacrifice.

Measurement of Urinary C5b-9 and Protein Excretion

Urine was collected overnight into a concentrated mixture of protease inhibitors and rat C5b-9 was assayed using an enzyme-linked immunoabsorbent assay that employed a monoclonal antibody to a neoantigen of rat C5b-9 (2A1) as a capturing antibody and a second monoclonal antibody to rat C6 (3G11) as a detecting antibody as described previously.[8] C5b-9 units were calculated using a previously defined zymosan-activated rat serum reference standard.[8,9] Protein excretion was measured on 24-hour urine specimens collected in metabolic cages using a sulfosalicylic acid method and whole serum standard as described previously.[9]

Direct Effect of CVF on C3c and C3d Deposits

To exclude a direct effect of CVF on the degradation of glomerular C3b, tissue sections from a PHN rat at day 5 were incubated for 2 hours at 37 C with the following solutions before being washed and stained for C3c and C3d: 1) PBS containing 40 mM EDTA (negative control); 2) PBS containing 40 mmol/L EDTA and 0.5 U CVF to assess C3b degradation by CVF; 3) 60% fresh rat plasma containing 40 mmol/L EDTA to assess C3b degradation by factor I (positive control); and 4) 60% fresh rat plasma containing 40 mmol/L EDTA and 0.5 U CVF to determine whether CVF had any additive effect on factor I–mediated C3b degradation.

Immunofluorescence Studies

Biopsies of kidneys were performed under ether anesthesia via a lateral flank incision. Hemostasis was achieved with Gelfoam (Upjohn, Kalamazoo, MI). The tissue was flash-frozen in Dry Ice–cooled isopentane and stored at −70 C until processed. Four-micron cryostat sections were cut and transferred to glass slides pretreated by rinsing in 50 μg/ml of poly-*l*-lysine (Sigma) in water and air dried. Sections were rinsed in PBS for 15 minutes with three buffer changes, and direct immunofluorescence was performed by incubation with 15 μg of FITC-labeled goat anti-rat C3 IgG or 10.5 μg of FITC-labeled rabbit anti-human C3d IgG. The molar fluorescein to protein ratio of the FITC-labeled anti-rat C3 was 1.37; the molar fluorescein to protein ratio of the rabbit anti-human C3d was 2.3. After one hour of incubation in a humid chamber at room temperature, the slides were washed for 15 minutes in PBS with three buffer changes, fixed in 1% paraformaldehyde (Kodak, Rochester, NY) in PBS, and mounted.

Quantitative Immunofluorescence Densitometry

Quantitative IF densitometry of biopsy material was performed using a Leitz 560 immunofluorescent microscope with visual output connected to a photomultiplier. All densitometry readings were done under oil immersion at ×640. The size of the densitometry field was fixed such that an average glomerular cross-section exceeded the field by 10 to 20%. Whole glomeruli which completely filled the defined field were identified by light microscopy scanning. The photomultiplier was calibrated by setting the glomerular fluorescence of a stock day 7 PHN biopsy (maximally intense for C3c and C3d) to an arbitrary value of 69 U. These stock biopsies were stained in every experiment for C3c and C3d. Five separate glomeruli were read in every section, and glomerular fluorescence for a section was then calculated as the mean of these five readings. Background values were obtained from normal rat kidneys stained with the same procedure and the values obtained were subtracted from all experimental readings. All tissues for each model, normals, and the standard biopsy were cut, stained, and analyzed in a single setting to minimize effects due to variations in tissue processing or in the intensity of the ultraviolet light source.

Statistical Analysis

Results of quantitative immunofluorescence densitometry measurements are expressed as means ± SD. Student's *t*-test was used to compare biopsies from CVF treated rats versus controls. P values <0.01 were considered statistically significant.

Results

Specificity of Polyclonal Antibodies to Rat C3 and Human C3d for the Fragments of Rat C3

The reactivity of the polyclonal antisera with fragments of rat C3 was tested using SDS-PAGE and immunoblotting (Figure 1). The polyclonal antibodies to rat C3 detected C3 in EDTA–plasma (Figure 1, *lane a*) and both C3b and C3c in CVF-activated serum (*lane b*). This antibody did not detect the C3d fragment (*lane b*). The polyclonal antibody to human C3d detected C3 in rat EDTA-plasma (*lane c*) and C3d in CVF-activated rat serum (*lane d*) but was not reactive with the C3c fragment.

Glomerular C3c and C3d Staining after Treatment with CVF in Vivo

The results of IF densitometry for C3c and C3d are presented in Table 1. In PHN, deposits were present exclusively in a typical subepithelial pattern along the capillary wall at all time points studied. Densitometric IF evaluation of initial biopsies obtained before CVF treatment on all PHN rats were strongly positive for C3c and C3d (Table 1). In PHN control rats staining intensity increased steadily for both C3c and C3d from days 2 to 7 after disease induction. Cationized IgG staining also increased from 24 to 48 hours in control rats but staining in con A was stable (C3c) or decreased (C3d) on the 2nd day.

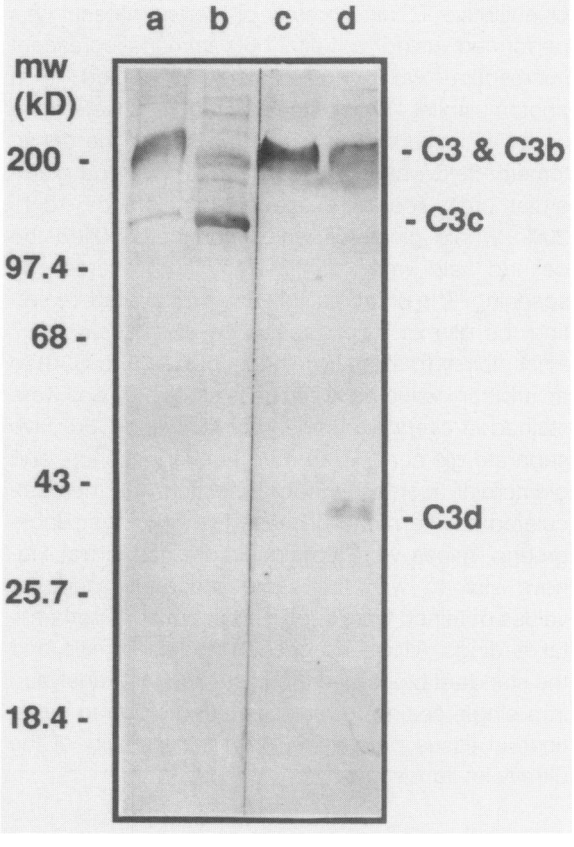

Figure 1. *Characterization of antibodies to rat C3c and C3d fragments by SDS-PAGE and immunoblotting. Rat EDTA-plasma* (lanes a *and* c) *and CVF-activated serum* (lanes b *and* d) *were separated by SDS-PAGE and transferred to nitrocellulose membranes. Antibody to rat C3 detects C3 in EDTA-plasma* (lane a) *and C3, C3b, and C3c in CVF-activated rat serum* (lane b). *No reaction with the C3d fragment is observed. The antibody to human C3d detects rat C3 in EDTA-plasma* (lane c) *and C3, C3b, and C3d in CVF-activated serum* (lane d). *There is no reactivity with rat C3c* (lane d).

All rats treated with CVF demonstrated a reduction in plasma C3 levels to less than 25% of normal 24 hours later. PHN rats treated with CVF on the 2nd and 4th day after disease induction demonstrated a marked reduction of glomerular C3c IF to <6% of control levels values not significantly different from background (Table 1, Figure 2), whereas rats treated on the 7th day were reduced to about 13% of controls (Table 1). In contrast, CVF treatment of PHN rats led to smaller decreases (27 to 62%) in C3d fluorescence 24 hours later at all three time points compared to the reductions seen with C3c (Table 1, Figure 2).

In cat-IgG, deposits were also predominantly subepithelial 24 hours after disease induction as previously reported.[12] In cat-IgG, glomeruli also stained positive for C3c and C3d before CVF treatment (Table 1). CVF decreased C3c fluorescence to background values in 24 hours compared with PBS-treated controls. In cat-IgG, C3d levels after CVF administration also decreased to 30% of controls treated with PBS but did not reach background values (Table 1).

In con A, immune deposits formed a band of electron density along the subendothelial surface of all capillary loops as previously described.[13] In con-A GN glomeruli also stained strongly for both C3c and C3d. After administration of CVF, C3c fluorescence was reduced to <10% of controls (Table 1). C3d levels after CVF dropped only to 52% of controls (Table 1).

Thus treatment with CVF in all three models resulted in almost complete disappearance of C3c within 24 hours. In contrast, C3d staining, although reduced after CVF treatment, remained consistently above background values at 24 hours in all three models.

Staining Intensity for C3c and C3d During the Heterologous and Autologous Phase of PHN

Serial evaluation of glomerular C3c and C3d IF during the heterologous and autologous phases of PHN was carried out on biopsies obtained every 3 days

Table 1 *Glomerular C3c and C3d Fluorescence Before and After CVF Treatment in Experimental Glomerulonephritis*

Fluorescence and model	Days*	Initial	After 24 hours		(% Control)
			Without CVF	With CVF	
C3c fluorescence					
Passive Heymann nephritis	2	20.6 ± 3.4	28.4 ± 3.6	−0.3 ± 1.5	0†
	4	26.8 ± 6.0	47.6 ± 8.4	2.6 ± 1.3	5.5†
	7	49.3 ± 7.3	59.6 ± 6.1	7.6 ± 1.7	12.7
Cat-IgG	1	16.5 ± 3.1	20.9 ± 2.3	−1.0 ± 0.5	0†
Con A anti–con A	1	37.8 ± 6.6	35.9 ± 5.1	2.6 ± 1.6	7.3†
C3d fluorescence					
Passive Heymann nephritis	2	28.2 ± 2.8	22.9 ± 3.7	13.6 ± 2.1	59.4
	4	38.5 ± 5.9	36.4 ± 6.1	14.1 ± 2.9	38.4
	7	42.7 ± 5.6	54.2 ± 4.7	39.5 ± 4.1	73.0
Cat-IgG	1	26.6 ± 2.4	34.5 ± 4.7	10.2 ± 2.9	29.7
Con A anti–con A	1	44.6 ± 8.8	36.0 ± 6.4	18.8 ± 2.9	52.3

* Days after disease induction.
† *P > 0.05 compared with background in normal rats.*

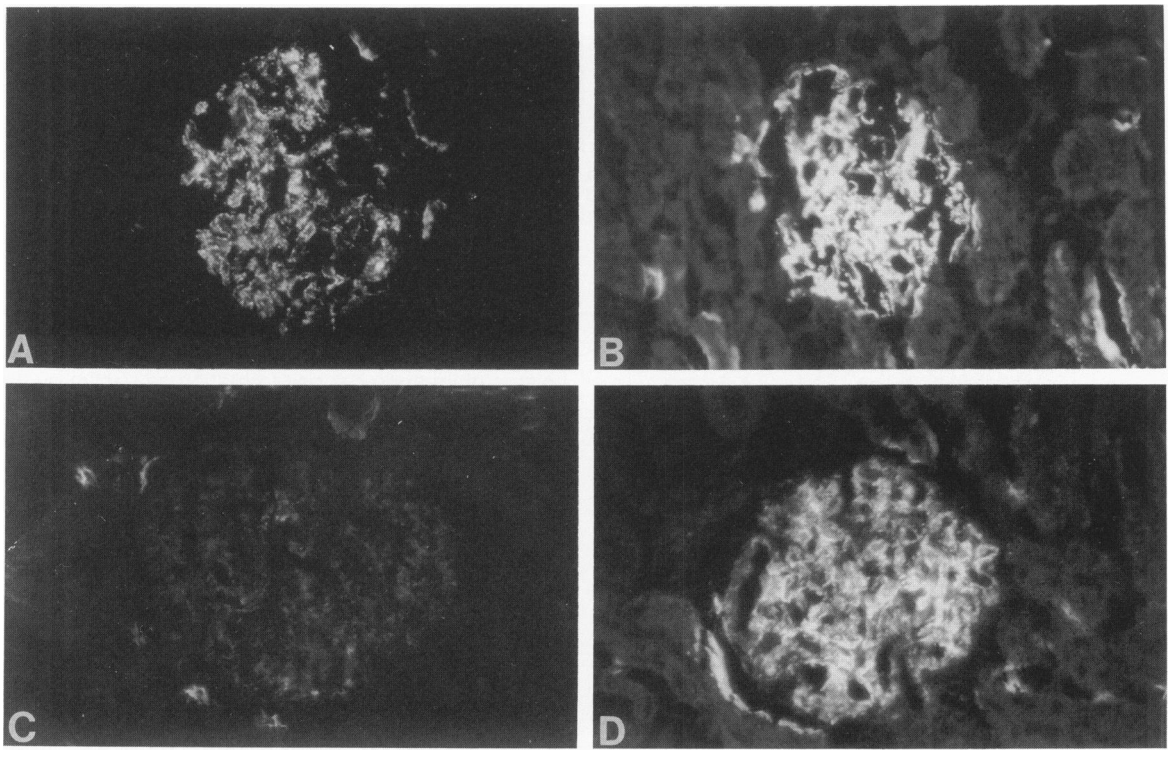

Figure 2. *Glomerular C3c* **(A)** *and C3d* **(B)** *immunofluorescence in PHN on day 4. Rats were then depleted of C3 with CVF and biopsied 24 hours later for C3c* **(C)**, *and C3d* **(D)**. *Abrogation of ongoing complement activation resulted in the rapid clearing of C3c, whereas C3d was unaltered at 24 hours.*

for 3 weeks after disease induction. Simultaneous urinary C5b-9 excretion was also measured at each time point. Urinary C5b-9 peaked at day 6 as reported previously[9] and then gradually diminished to undetectable levels by day 18, and remained negative thereafter (see Figure 4), indicating cessation of the autologous phase.[9] Urinary protein excretion was also increased on day 6, peaked by day 9, and remained elevated thereafter as previously reported[9] (see Figure 4). IF densitometric readings for C3c paralleled the gradual rise and fall of urinary C5b-9, although IF for glomerular C3c lagged behind C5b-9 excretion by 3 days (Figures 3 and 4). C3c slowly increased and reached maximum intensity at day 9 (Figure 3). Thereafter, glomerular C3c slowly decreased to reach baseline levels by day 23 (Figures 3 and 4). The immunofluorescence staining for C3d in glomeruli also gradually rose, and peaked at

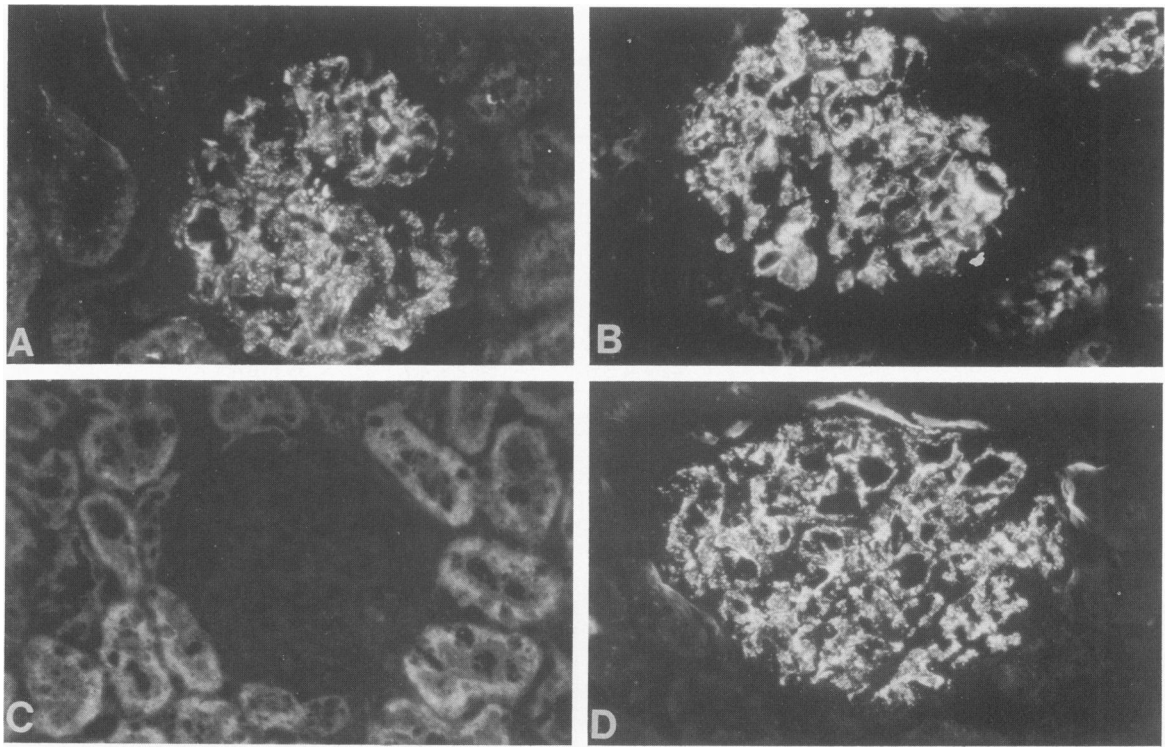

Figure 3. *Glomerular C3c and C3d immunofluorescence during the autologous phase of PHN.* **A**: *C3c fluorescence on day 9 (22.0 ± 7.4 U).* **B**: *C3d fluorescence on day 9 (44.1 ± 10.8 U).* **C**: *C3c fluorescence on day 23 (5.5 ± 2.1 U).* **D**: *C3d fluorescence on day 23 (31.3 ± 12.1 U).*

day 9. In contrast to the urinary C5b-9 and staining for C3c, however, C3d staining remained prominent for the duration of the study (Figures 3 and 4).

Evaluation of the Direct Effect of CVF on Glomerular C3c and C3d Staining

The incubation of sections from PHN rats with CVF had no effect on IF densitometry for C3c or C3d compared with PBS controls (Table 2). When tissue sections were incubated with EDTA–plasma containing factor I, C3c fluorescence was reduced by 30% in 2 hours. The addition of CVF to EDTA–plasma did not alter this factor I-mediated C3b degradation (Table 2).

Discussion

This study is the first to examine the kinetics of C3 deposition and degradation in experimental glomerular disease. The results suggest that very clinically useful information on disease actually may be obtained from assessment of C3 staining in human glomerulonephritis. The complement-depletion studies with CVF demonstrate that cleavage of C3b to C3c and C3d occurs within 24 hours of the cessation of ongoing complement activation. This appeared to be true regardless of the quantity of the C3b deposited as indicated by the fact that C3c staining disappeared almost as completely in PHN rats treated with CVF late on day 7 as in those treated with CVF much earlier on day 2. It was also independent of mechanisms of immune deposit formation as shown by the similar results in the PHN (induced with antibody to an endogenous antigen) and cat-IgG (induced with antibody to an exogenous antigen) models. C3b degradation was further independent of the site of immune deposit formation as illustrated by similar results obtained when immune deposits were primarily subepithelial (PHN, cat-IgG) or subendothelial (con A) in location. Thus, our findings imply that positive staining for C3, as detected with an antibody directed at C3c, documents significant complement activation within 24 hours and therefore indicates ongoing, immunologically mediated disease activity. These findings are in accord with previous studies by Noble et al[15] demonstrating disappearance of C3c staining within 3 days after CVF administration to rats with chronic serum sickness.

Ongoing complement activation could result from continued deposition of antibody and formation of complement-activating glomerular immune complex deposits, continued complement activation by previously formed or deposited immune complexes or (in PHN) by complement activation by damaged glomerular epithelial cells converted to alternate pathway activators by deposition of anti-Fx1A

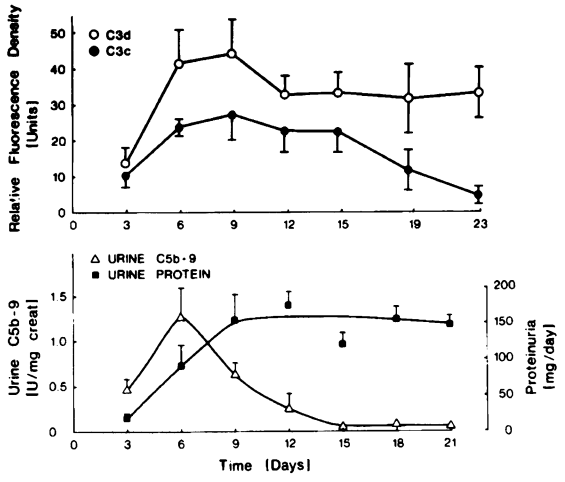

Figure 4. *Glomerular C3c and C3d fluorescence during the heterologous and autologous phase of PHN* (top) *and urinary C5b-9 and protein excretion* (bottom). *C3c fluorescence decreased in parallel with the decline in urinary C5b-9, reflecting cessation of the autologous phase and of ongoing immune deposit formation, whereas C3d and urine protein excretion remain stable. Data on urinary C5b-9 and protein excretion adapted from reference 9.*

antibody.[16] Since therapeutic initiatives are generally directed at suppressing the antibody response, C3 staining would be of the most clinical value if C3 reflected complement activation by new immune deposits. The sequential study of C3c and C3d staining in unmanipulated PHN rats was designed to address this issue. Our 23-day study in unmanipulated PHN rats suggests that ongoing C3 activation does reflect primarily new immune deposit formation rather than ongoing complement activation by old deposits or by damaged cells converted to alternate pathway activators.[16] Thus, previous studies have demonstrated that urinary C5b-9 excretion is a sensitive measure of ongoing deposition of both heterologous and autologous antibody in this model.[8,9] When C3c staining was correlated with urinary C5b-9 excretion in PHN, we found that staining consistently disappeared within 3 days of the cessation of antibody deposition despite persistent IgG deposits that have apparently now lost their ability to fix further complement. The difference between the less than 24 hours required for C3c to disappear after CVF and the 72 hours between cessation of urinary C5b-9 excretion and C3c disappearance in unmanipulated PHN rats may reflect some deposit formation below the threshold that can be detected by urinary C5b-9 excretion, some C3 activation by existing deposits, or some complement activation by damaged cells.[16,17] However, at least in experimental membranous nephropathy (MN), positive C3 staining would appear to reflect primarily complement activation secondary to ongoing immune deposit formation.

Table 2 *Effect of Incubation with CVF in PBS or in Rat Plasma on Glomerular C3c and C3d Fluorescence in Vitro*

Reagents	C3c*	C3d*
PBS-EDTA (control)	50.9 ± 5.9	60.6 ± 12.5
PBS-EDTA-CVF	47.8 ± 7.21	56.2 ± 6.73
Plasma-EDTA	35.9 ± 2.8	52.6 ± 5.2
Plasma-EDTA-CVF	35.5 ± 5.32	54.5 ± 8.14

* Quantitative IF densitometry (mean ± SD) after 2 hours of incubation on sections of PHN kidney obtained 5 days after disease induction.

Our study does not address the mechanism of C3b removal from glomeruli. C3b is presumably attached to glomerular cell surfaces (or bound Igs) and could be degraded by factor H or I to covalently bound C3d, and C3c which is released, or could be eliminated by cellular mechanisms such as capping, shedding, or endocytosis.[18] The persistence of C3d compared with C3c suggests that proteolytic cleavage by factors H and I is the principal mechanism involved. If cellular mechanisms were primarily responsible for this removal, the elimination of C3c and C3d would be predicted to occur simultaneously. This finding contrasts with the observation of Quigg et al[16] that the stabilized C3 convertase C3b BbP formed on cultured rat GEC *in vitro* after anti-Fx1A antibody deposition is not influenced by fluid phase regulatory proteins. Jepsen et al[19] have suggested that degradation of C3b to C3c and C3d proceeds efficiently only in the presence of CR1 receptors, and Vedeler et al[20] have documented the ability of CR1 to provide the necessary co-factor activity for factor I-mediated degradation of C3b to C3c and C3d. The presence of CR1 on human glomerular epithelial cells is well established.[20–22] In the rat, conclusions have differed with some investigators reporting detectable levels of CR1 on the normal rat glomerular epithelial cell[22,23] and others reporting negative results.[24] However, Kerjaschki has recently described up-regulation of the glomerular epithelial cell CR1 receptor in the PHN model (personal communication). Thus CR1 receptors are likely to be present under the conditions of the studies in PHN performed here.

The human glomerular disease to which the studies of PHN can be most easily related is idiopathic membranous nephropathy. In experimental membranous nephropathy, proteinuria has been well shown to persist for weeks to months after immunological disease activity ceases.[9,25,26] This phenomenon, as well as the very slowly progressive nature of the disease, makes assessment of disease immunologic activity (and therefore appropriate selection of patients for intense immunosuppressive therapy) particularly difficult using currently available clinical criteria. The recent reports by us[27] and others[28] that

urinary C5b-9 excretion is elevated in a subset of patients with MN may finally provide a good measure of immunologic disease activity. However, the urinary C5b-9 excretion assay must be correlated with excretion of native complement components and is not widely available. If staining for C3c in biopsy tissue can provide similar data, as the present study suggests, this could be of considerable clinical value in selecting appropriate patients for immunosuppressive therapy. Previous studies of glomerular C3 IF in MN have given widely differing results, with most reports suggesting positive staining in about 50 to 90% of patients.[29–31] However, some studies have reported largely negative results.[32,33] When antibodies specific for C3c and C3d were utilized, C3d was found in all patients and C3c in about one-half.[29] Moreover, patients with C3c staining appeared to have more severe disease,[29] an observation also made in patients exhibiting elevated urinary C5b-9 excretion.[27] Although some of the variability in C3 staining in MN may reflect differences in antibody specificity of the anti C3 antibodies used, most anti-human C3 antibodies react primarily with C3c, as did the antibody utilized in this study. Thus, the IF data in human MN is quite consistent with our findings in experimental MN. It supports the hypothesis that only a subset of patients presenting with nephrotic syndrome and MN that undergo biopsy actually have active disease with ongoing immune deposit formation as indicated by positive C3c staining. The present results provide strong support for a clinical study to attempt to assess the relationships among C3c staining in human MN, other measures of disease activity such as urinary C5b-9 excretion, long term course, and response to therapy. It should be noted that a similar interpretation of C3c staining may apply as well to other antibody-mediated glomerular lesions such as IgA nephropathy.[34]

In summary, our data suggest that positive glomerular staining for C3 with antibody specific for C3c documents very recent complement activation, likely due to ongoing immune deposit formation in glomeruli. Glomerular C3 IF correlates closely with other independent measures of immunologic disease activity in experimental MN. In contrast, C3d staining is usually persistent and does not closely parallel disease activity. These findings suggest that positive C3 staining using antibodies specific for the C3c fragment in human disease may provide a more sensitive measure of the activity of antibody-mediated glomerular disease at the time of biopsy than can be obtained from other currently available clinical or morphological criteria. For example, a correlation between hematuria and glomerular C3 staining has been noted in experimental IgA nephropathy.[35]

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